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Predicting the Risk of Tumor Occurrence under the Effect of Small Doses of Carcinogens

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Basic methodological approaches were formulated for determining the permissible levels of carcinogens in man's environment on the basis of current experimental oncological data dealing with the interaction of the organism and carcinogenic compounds. The tumor is proposed as a specific index of the harmfulness criterion of carcinogenic action. Under these conditions, both the frequency and the development time of tumors must be considered.

Results are given of an experimental study of the carcinogenic activity of various doses of benz[a]pyrene (0.005, 0.02, 0.1, 0.5, and 2.5 mg) on rats by using a tenfold intratracheal administration. On the basis of mathematical models of the obtained dose-time-effect relationship, the risk of cancer occurrence due to small carcinogen doses is predicted. Small doses were not tested in the experiment.

On the basis of the data obtained in the experiment, a maximally permissible concentration of benz a pyrene in the ambient air can be determined. A benz[a]pyrene dose of 0.02 mg is recommended as the basis of the calculation. The effect of this dose is shown at time periods longer than the limits of human life.

Quantitative criteria of the action of chemical carcinogenic compounds serve as the basis for establishing the degree of danger they pose and safety levels.

Since it is still not clear at this time whether an effect threshold exists for carcinogenic substances, the real task is to determine the specific effect which may appear beyond the limits of human life. This would serve as a tolerable carcinogen dose. This can be done by developing a model for the quantitative relationship of the effect of a carcinogenic agent under oncologic experimental conditions, with subsequent extrapolation of the data to man.

This includes: (1) study of various doses of carcinogen in the range of optimal to minimally effective and maximumly ineffective doses with observation of the animals during their life span; (2) mathematical modelling of the carcinogen dose–blastomogenic effect and the relationship of carcinogen dose to time of effect; (3) the quantitative prediction of the theoretical risk of appearance of a tumor as a result of the action of small doses of carcinogen during the time period beyond the limit of the maximum duration of animal life; (4) calculation of maximum permissible concentration (MPC) of carcinogen in the appropriate elements of the environment.

Since it was not possible to conduct the detailed experimental studies in accord with the general requirements of experimental oncology, let us examine some of the principal considerations.

One of the most complex and the least studied problems is that of the extrapolation of data obtained on animals to humans. For this reason, in conducting studies on the establishment of standards for carcinogens, it is important to make test conditions as realistic as possible, in particular with respect to the introduction of the carcinogenic agent into the organism.

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This involves a second problem with the suitability of experimental models for forms of tumors observed in the pathology of man. The complexity of this problem must be stated, since it is known that tumor form and localization depend not only on the species of laboratory animal and the mechanisms of transformation of carcinogenic agent in each of the species, but also to a great extent on the carcinogen dose and the conditions and duration of carcinogen contact with organs and tissues. Thus, for example, in our studies we observed that the dose of benzpyrene has an effect on the histological type of lung cancer. This is shown by the dominant development of epidermoid cancer with the administration of large doses and the tendency towards the development of adenocarcinoma at lower doses (*1*).

Pylev and Grisyute, each, administered identical doses of dimethylbenzanthracene (DMBA) to Wistar strain rats. Lung cancer resulted in the same number of instances; approximately 30% of the animals developed lung cancer. Nevertheless in Pylev's tests, epidermoid cancer predominated with keratinization, and the premalignant changes consisted of metaplasia, while in Gritsyute's tests, the tumors appeared later and were primarily represented by undifferentiated adenocarcinomoid forms. This is most likely explained by the difference in the conditions of administration: Gritsyute used long administration periods with large intervals, while Pylev used "shock" doses with small intervals which evoked metaplasia (*2*).

Turusov (*3*) observed the effect of carcinogen dose on the histologic structure of cancer in the case of cutaneous carcinogenesis. Arkhipov (*4*) produced tumors in the body of the stomach of mice during experimental disruption of its functional state (reduction of secretion) by introducing ephedrine chloride. Introduction of a carcinogen with food results in damage to the forestomach due to longer contact with the mucosa of the forestomach while the entrance of the same carcinogen into an empty stomach also produces tumors in various sections of the intestines. The given data show the necessity of taking into consideration the entire blastomogenic reaction of an organism as the response to the action of a carcinogenic factor.

The use in experiments of animals with various spontaneous tumor levels remains a debatable question. It would seem advisable to use animals with high cancer susceptibility in determining the effect of small carcinogen doses, since as Shabad (*5*) points out, spontaneous tumors in themselves attest to a sensitivity to blastomogenic substances.

In conjunction with this, our studies (*6*) showed that minimally effective doses induce single tumors at the end of the natural life of an animal. This in time coincides with the development of spontaneous tumors. Thus against this background, it is not possible to differentiate the induced tumors.

The increase in the frequency of spontaneous tumors in in-bred animals under the effect of a carcinogenic factor depends entirely on the genetic properties of the animal strain selected for testing (*7*).

At the same time, random-bred animals are genetically heterogeneous. This makes them a closer approximation to the human population and on this basis it is more advisable to use such animals in this type of experiment.

So far as the duration of exposure is concerned, there undoubtedly is a need for further scientific elaboration. Based on our experience, two types of procedures are possible and each one has its own advantages.

Procedure 1 consists of the administration of standard carcinogen over the entire lifespan of animals. This method is optimal with respect to public health applications, since it approximates real conditions, but it is exceedingly complex and laborious with respect to implementation.

Procedure 2 consists of administration of a carcinogen to several groups of animals at different fractionations. Results obtained in such an experiment provide considerably more information and can serve as a basis for a mathematical model of dose–time effect. This will make it possible to use these results to predict the effect of the chronic action of a carcinogen on the basis of the results of a short-term experiment.

On the basis of these data, there emerges the possibility of determining the coefficient of cumulation, which is an important index of the characteristics of the biological effect of a carcinogen.

Another important problem is the determination of criteria of harmful activity. In spite of the large amount of information available by experimental oncology on correlations between the carcinogenic factor and the organism and subsequently between tumor and the organism, up to the present there are no specific indices for defining the initial stages of the neoplastic process before appearance of a tumor.

Although the specificity of the tumor process is ordained as early as the first stages of carcinogenesis, still it is difficult to determine on a

Table 1. Appearance of tumors in random-bred rats after a tenfold intratracheal administration of various total benzpyrene doses.

Benzpyrene dose, mg	Number of animals receiving total dose	Epithelial tumors of the lungs: Malignant %	Epithelial tumors of the lungs: Malignant Time to occurrence, months	Epithelial tumors of the lungs: Benign %	Epithelial tumors of the lungs: Benign Time to occurrence, months	Reticulosarcoma of the lungs %	Reticulosarcoma of the lungs Time to occurrence, months	Tumors localized elsewhere %	Tumors localized elsewhere Time to occurrence, months
25.0	40	32.5	14.4	37.5	12.8	10.0	15.5	5.0[a,b]	16.0
2.5	25	21.4	17.1	14.3	17.3	7.1	17.2	3.6[c]	22.5
0.5	32	15.7	25.1	12.5	19.1	—	—	3.1[b]	24.3
0.1	28	—	—	14.4	27.0	—	—	3.6[c]	26.7
0.02	16	—	—	—	—	—	—	—	—
0.005	17	—	—	—	—	—	—	—	—
Control	21	—	—	—	—	—	—	9.5[b,d]	24.0

[a]Cancer of the bladder.
[b]Sarcoma of the liver.
[c]Fibroadenoma of the mammary gland.
[d]Lymphosarcoma of the mediastinum.

practical level whether initial changes are premalignant or bear some other nonspecific nature, for example inflammatory. Therefore at this stage, we should consider the formation of tumors as a reliable index of the specific action of carcinogens. Under these conditions it is both necessary to take into consideration the frequency and time to appearance as well as the fact that tumor frequency is determined both by the number of inducible neoplasms and by a higher level of spontaneous tumors.

The hypotheses presented are used as the basis in predicting risk from the effect of small doses of benzpyrene. We studied a number of benzpyrene doses in the 0.005 to 25 mg range with tenfold intratracheal administration to random-bred white rats (Table 1).

Under these conditions tumors were obtained in the lungs and other organs of the animals. The long-term neoplasms were singular and diverse with respect to histologic type. This made it possible to study them as spontaneous and independent of the action of benzpyrene. Epithelial tumors were observed in the lungs. As is known, this type of tumor is not encountered as a spontaneous manifestation in rats (*8–11*) and also the connective tumors of the reticulosarcoma type. The latter type significantly exceeded the spontaneous level which is 0.8–1.3% according to the data of Ird and Konoplev (*9*) and A. I. Vysamyae (*11*).

On the basis of the data in Table 1, the number tumor-bearing animals diminishes as the benzpyrene dose is reduced. The time for appearance of a neoplasm increases, and as a result, the number of spontaneous lung tumors also increases. A shortening of the latent period for lung tumor development occurs only under the effect of large benzpyrene doses. Small doses of the compound resulted in including only epithelial neoplasms. In this experiment, the total dose of 0.1 mg is minimally effective and 0.02 and 0.005 mg are ineffective.

In analyzing the data, we must say a few words about the given conditionality or dynamicity which is dependent on the experimental conditions associated with such values as the minimally effective and ineffective dose. Thus for example, a benzpyrene dose of 0.1 mg administered once and five times does not produce tumors (Table 2), but a tumorigenic effect of this dose was shown after tenfold fractional introduction.* One question that remains unclear is concerned with the potential danger of the maximally noneffective dose of 0.02 mg obtained in our experiment. How potentially dangerous is this dose if it is administered daily throughout the life of an animal, rather than over a 10-month period as we did it?

Finally, let us look at sensitivity. The fact is that our studies were conducted on relatively small

*Editor's note: X-fold (fractional) introduction appears to indicate that the a given total dose was divided into x portions, administered in x doses.

Table 2. Appearance of lung tumors as a function of the number of doses of benzpyrene.

Total dose, mg	Incidence of lung tumors, %						
	1 dose		5 doses		10 doses		36 doses
	Experimental	Theoretical[a]	Experimental	Theoretical[a]	Experimental	Theoretical[a]	(theoretical[a])
25.0	30.0	14.7	55.5	55.1	80.0	79.8	99.6
10.0	27.2	10.2	—	41.5	—	65.6	97.9
2.5	13.0	5.4	—	24.3	42.8	42.6	86.5
0.5	5.9	3.3	—	5.5	28.2	28.4	70.0
0.1	0.0	0.9	0.0	4.9	14.4	9.4	30.0
0.02	—	0.1	0.0	0.9	0.0	2.1	6.9

[a]Theoretical values calculated according to the formula: $y = 100(1-e^{-ar})$, where y is the risk of tumors in animals (in %); a is an index of risk increase, determined according to the graph dependence $a = f(D)$, r is the number of portions into which total dose is divided.

groups of animals. Under these conditions, there is great variation in the sensitivity of the individuals constituting the group to carcinogenic substances. For this reason, instead of a sharply expressed threshold, the dose–response curve can have a long tail. Under certain assumptions, this can be determined through the approximation of the experimental data by using mathematical models. We did this by using the logarithmic function equation, eq. (1), plotted in Figure 1.:

$$Y = 10 \ln \left[(X_n/X)\right] + 1 \qquad (1)$$

where Y is the percentage of animals with tumors, X_n is the carcinogen dose (in mg), and X is the maximally noneffective dose (in mg) and also by using the exponential equation [eq. (2)] plotted in Figure 2:

$$Y = 100\,(1 - e^{-ax}) \qquad (2)$$

where Y is the percentage of animals with tumors, x is the carcinogen dose (in mg), and a is the coefficient of proportionality and dimensionality; a = 1.555 when the exponential curve passes through the 0.1 mg point and a = 0.064 when the exponential curve passes through the 25 mg point.

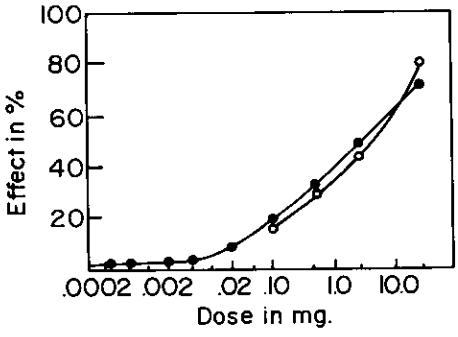

FIGURE 1. Frequency of lung tumors as a function of the benzpyrene dose administered to the respiratory tract of random-bred rats (functional logarithmic equation): (o) experimental; (•) points computed with the function $Y=10\ln[(X_n/X)] + 1$.

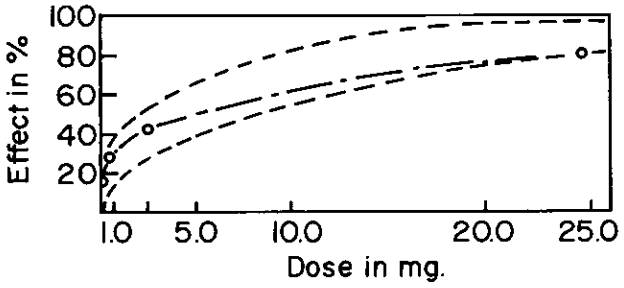

FIGURE 2. Frequency of lung tumors as a function of the benzpyrene dose administered to the respiratory tract of random-bred rats [functional exponential relationship, $Y=100(1-e^{-ax})$].

As can be seen, the quasithreshold includes a broad range of benzpyrene doses (Fig. 1). Those benzpyrene doses which turned out to be ineffective in the experiment may under certain conditions induce neoplasms. We utilized a supplementary dose–time relationship in order to establish a better basis for the selection of the allowable benzpyrene dose. To calculate the theoretical occurrence time of the first tumor T as function of small benzpyrene doses d [eq. (3)] was used:

$$T=(a/d)+B \qquad (3)$$

where a = 1.021 and B = 16.79.

Table 3 shows the computed occurrence time for the initial (first) tumor. As can be seen from Table 3, the latency period for the initial tumor is 27 months in the case of a 0.1 mg dose tested in the experiment. The occurrence time for the initial tumor resulting from a dose two times less than the above

Table 3. Calculated time for appearance of the first lung tumor after administration of various total benzpyrene doses in ten portions intratracheally.

Benzpyrene dose, mg	Time of tumor occurrence, months
0.1	27.0
0.05	38
0.02	67.9
0.01	118.9
0.005	221.0
0.002	527.3

is predicted at 38 months, i.e., towards the end of the animal's lifespan. Smaller doses induce initial neoplasms past the lifespan limits of the animal.

We recommend a 0.02 mg dose as the permissible dose and consider that it is possible to use this dose for calculation in the dose–concentration formula.

Thus despite the given theoretical risk of tumor occurrence resulting from the effect of small carcinogen doses, there exists the possibility of determining such doses whose effect may manifest itself only past the lifespan limits of the animal.

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